Accumulation of hypoxia-inducible factor-1α protein and its role in the differentiation of myeloid leukemic cells induced by all-trans retinoic acid

Jing Zhang,[1] Li-Ping Song,[1] Ying Huang,[2] Qian Zhao,[1,2] Ke-Wen Zhao,[2] and Guo-Qiang Chen[1,2]

[1]Institute of Health Science, Shanghai Institutes for Biological Sciences of Chinese Academy of Sciences (SIBS)-Shanghai Jiao-Tong University School of Medicine (SJTU-SM), Shanghai and [2]Department of Pathophysiology, Key Laboratory of Cell Differentiation and Apoptosis of the Chinese Ministry of Education, SJTU-SM, Shanghai, China

ABSTRACT

Background

The clinical activities of *all-trans* retinoic acid in the treatment of acute promyelocytic leukemia, a unique subtype of acute myeloid leukemia, have triggered extensive studies aimed at defining the mechanisms by which this compound induces differentiation of leukemic cells. Recent studies show that hypoxia-inducible factor-1α (HIF-1α) contributes to the differentiation of acute myeloid leukemia cells via transcriptional activity-independent mechanisms. We investigated whether *all-trans* retinoic acid affects HIF-1α protein and whether this has a role in *all-trans* retinoic acid-induced differentiation.

Design and Methods

The acute myeloid leukemia cell lines NB4 and U937 were treated with *all-trans* retinoic acid, and HIF-1α/HIF-1β mRNA and proteins were measured respectively by real-time quantitative reverse transcriptase polymerase chain reaction and western blotting. To investigate the role of HIF-1α in *all-trans* retinoic acid-induced differentiation, NB4 cells, U937 cells, U937 cells in which HIF-1α was induced by the withdrawal of tetracycline and U937 cells with stable expression of specific short hairpin RNA against HIF-1α, Runx1, C/EBPα and PU.1, were treated with *all-trans* retinoic acid and/or the hypoxia-mimetic agent cobalt chloride ($CoCl_2$). Cellular differentiation was evaluated by morphological criteria and myeloid differentiation antigens.

Results

all-trans retinoic acid rapidly increased endogenous and inducible expressed or $CoCl_2$-stabilized HIF-1α protein in leukemic cells under normoxia. Importantly, suppression of HIF-1α expression by specific short hairpin RNA partially but significantly inhibited *all-trans* retinoic acid-induced differentiation of the U937 cell line. Reciprocally, the differentiation induced by *all-trans* retinoic acid was significantly enhanced by conditional HIF-1α induction and HIF-1α-stabilizing $CoCl_2$ treatment. Furthermore, knock-down of *PU.1*, *Runx1* and *C/EBPα*, three transcriptional factors crucial for normal hematopoiesis, greatly inhibited the differentiation cooperation of *all-trans* retinoic acid and HIF-1α induction.

Conclusions

This work provides the first demonstration that HIF-1α, a protein rapidly responsive to *all-trans* retinoic acid, plays a role in *all-trans* retinoic acid-induced differentiation of leukemic cells. These observations shed new light on the molecular mechanisms underlying *all-trans* retinoic acid-induced differentiation of acute myeloid leukemia cells.

Key words: hypoxia inducible factor-1α, *all-trans* retinoid acid, differentiation, leukemia.

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Correspondence:
Guo-Qiang Chen,
No. 280, Chong-Qing South Rd,
Shanghai 200025, China.
E-mail: chengq@shsmu.edu.cn

Introduction

Retinoic acid and the derivatives (retinoids) are promising anti-cancer agents endowed with both therapeutic and chemopreventive potential.[1] An outstanding example of the use of retinoids is the successful differentiation of leukemic cells induced by *all-trans* retinoic acid (ATRA) in patients who suffer from acute promyelocytic leukemia (APL),[2] a unique subtype of acute myeloid leukemia (AML), which harbors the chromosome translocation t(15;17) and expresses the fusion protein PML-RARα (for *p*ro*m*yelocytic *l*eukemia-*r*etinoic *a*cid *r*eceptor α).[3] The important *in vitro* and *in vivo* activities of ATRA have triggered extensive studies aimed at defining the mechanisms by which this compound induces differentiation with growth arrest and apoptosis of AML cells.[4] It has been established that pharmacological doses of ATRA induce degradation of the PML-RARα protein,[5] and reverse the dominant-negative effects on functions of the wild type PML and RARα proteins of the PML-RARα oncoprotein, which interacts with transcriptional co-repressors such as the nuclear co-repressor -histone deacetylase complex in an ATRA-sensitive manner, blocking the activation of RARα target genes.[6] ATRA induces gene transcription via RAR/RXR (retinoid X receptor) heterodimer-bound retinoic acid responsive elements that are present in the promoters of retinoid-responsive genes, and ultimately results in the production of proteins that regulate differentiation and induce cell-cycle arrest of leukemic cells. Using unique cellular and transgenic APL models and fast-developing molecular genetic, proteomic and functional genomic approaches, many ATRA target genes have been discovered in the past decade.[7,8]

On the other hand, there is increasing evidence to suggest that additional signaling pathways are activated in ATRA-treated cells. These signaling molecules include Stat-proteins, tyrosine kinases, cAMP/protein kinase A,[9] calcium-dependent signaling and mitogen-activated protein kinases.[10] Several transcription factors with their cooperative effects, such as CCAAT/enhancer-binding proteins (C/EBP), Runx1 (originally named acute myeloid leukemia 1, AML1) and PU.1, also exert critical roles in hematopoietic cell differentiation.[11] Dysregulation and/or mutations of these factors have been found in many types of leukemia resulting in differentiation blockage.[12] These transcriptional factors also contribute to ATRA-induced differentiation of AML cells.[13,14]

Hypoxia-inducible factor 1 (HIF-1), composed of the constitutively expressed HIF-1β subunit and the highly regulated HIF-1α subunit,[15] is a master transcriptional regulator in cellular responses to hypoxia, participating in many physiological and pathological processes.[16] Recently, we reported that HIF-1α-stabilizing hypoxia and hypoxia-mimetic agents, such as cobalt chloride ($CoCl_2$) and desferrioxamine, can also cause the differentiation of AML cells *in vitro* and *in vivo*.[17,18] Conditional HIF-1α induction also triggers differentiation of leukemic U937 cells in a transcriptional activity-independent manner.[19] Furthermore, HIF-1α protein can interact with C/EBPα and Runx1 proteins and increase their transcriptional activities.[17,20,21] Kim *et al.* found that Tiron, an antioxidant and non-toxic chelator widely used to alleviate acute metal overload, induces differentiation of human HL-60 leukemic cells through increased expression of HIF-1α.[22] In this study we investigated the effects of ATRA on HIF-1α protein in AML cell lines.

Design and Methods

Cell lines and differentiation assay

The human APL cell line NB4 and the monocytic leukemic cell line U937 were incubated in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA), supplemented with 10% fetal bovine serum (FBS, HyClone, Logan, UT, USA). $U937T^{empty}$, $U937T^{clone}$, and $U937T^{pool}$ cells[19] were cultured in RPMI-1640 medium supplemented with 10% FBS, 1 μg/mL of tetracycline and 0.5 μg/mL of puromycin (Sigma-Aldrich). All cell lines were cultured in 5% CO_2/95% air in a humidified atmosphere at 37°C. In all experiments, cell viability exceeded 95%, as determined by a trypan-blue exclusion assay. For morphological characterization, cells were collected onto slides by cytospinning (Shandon, Runcorn, UK), stained with Wright's stain and examined by light microscopy (Olympus, BX-51, Tokyo, Japan). The differentiation antigens CD11b and CD14 were measured using fluorescein isothiocyanate (FITC)-labeled anti-CD11b or anti-CD14, with isotype controls, by flow cytometry (Beckman-Coulter, Miami, FL, USA). The nitroblue tetrazolium (NBT) reduction test was performed as previously described.[23]

Real-time quantitative reverse transcriptase polymerase chain reaction

Total cellular RNA was extracted from cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reverse transcription was performed using the cDNA synthesis kit according to the manufacturer's instructions (Promega, Madison, WI, USA). Real-time quantitative RT-PCR was performed with the double-stranded DNA dye SYBR Green PCR Master Mixture Reagents (Applied Biosystems, Warrington, UK) on an ABI PRISM 7900 system (Perkin-Elmer, Torrance, CA, USA). Data were analyzed as previously reported.[24] The specific primers used for β-actin, HIF-1α, HIF-1β, macrophage (M)-colony-stimulating factor receptor (CSFR), granulocyte (G)-CSFR and granulocyte-macrophage (GM)-CSFR have been reported previously.[19]

Short hairpin (sh)RNA design and transfection

Pairs of complementary oligonucleotides against HIF-1α, C/EBPα, Runx1 and PU.1 were synthesized (Invitrogen, Shanghai, China), annealed and ligated into the mammalian expression vector, pSilencer 3.1-H1 neo, according to the manufacturer's instruction (Ambion, Austin, TX, USA). The target sequence for

PU.1 was 5'- GAAGATCCGCCTGTACCAG-3'. Other targeted sequences have been described previously.[19,20] These shRNA-containing vectors and the negative control pSilencer neo vector were transfected into U937 cells and U937T^{clone} cells using the Gene Pulser Xcellt Eukaryotic System (Bio-Rad, Hercules, CA, USA). Forty-eight hours later, 600 μg/mL of G418 (Sigma-Aldrich) were added to select the stably transfected cells.

Western blot

Cell lysates were fractionated on a 10% sodium dodecyl sulfate–polyacrylamide gel and immunoblots were performed with primary antibodies against HIF-1α and HIF-1β (BD Transduction, San José, CA, USA), C/EBPα, Runx1 and PU.1 (Santa Cruz, CA, USA). As necessary, blots were stripped and reprobed with anti-β-actin (Merck, Darmstadt, Germany) or lamin B (Santa Cruz) antibody as a loading control.

Statistical analysis

Student's t-test was used to evaluate differences between treatments. A p value of less than 0.05 was considered statistically significant.

Results

ATRA increases HIF-1α protein but not its mRNA in leukemic NB4 and U937 cell lines

In order to investigate whether ATRA affects expression and/or stabilization of HIF-1α protein, the AML cell lines NB4 and U937 were treated with increasing concentrations of ATRA for 24 hours, and HIF-1α mRNA and protein levels were assessed, respectively, by real-time quantitative RT-PCR and western blotting. As depicted in Figure 1A, ATRA increased HIF-1α protein in a dose-dependent manner in both cell lines cultured in normal air. A time course analysis in NB4 cells treated with 10^{-6} M of ATRA revealed that HIF-1α protein began to be accumulated by 6 hours, reached peak levels at 24 hours, and then gradually decreased after 48 hours. However, ATRA-treated U937 cells showed a time-dependent and lasting increase of HIF-1α protein over the periods tested (Figure 1B). ATRA also decreased HIF-1β protein, which is essential for the transcriptional activity of HIF-1α,[15] in dose- and time-dependent manners in NB4 cells but not in U937 cells (Figure 1A and B). Of note, ATRA failed to change the levels of HIF-1α and HIF-1β mRNA in either NB4 or U937 cells (Figure 1B).

Suppression of HIF-1α expression by shRNA partially but significantly blocks ATRA-induced differentiation of myeloid leukemic cells

The ability of ATRA to induce differentation was more potent in NB4 cells than in U937 cells (Figure 1A and *data not shown*). In line with this, HIF-1α protein was accumulated to a greater extent in NB4 cells than in U937 cells exposed to the same concentrations of ATRA (Figure 1A). Furthermore, 10^{-7}M and especially 10^{-6}M of ATRA induced greater differentiation and HIF-1α protein-accumulating effects than 10^{-8}M of ATRA in these two cell lines (Figure 1A). This consistency between HIF-1α accumulation and induction of differentiation led us to investigate whether HIF-1α accumulation also contributes to ATRA-induced myeloid cell differentiation. We, therefore, transfected U937 cells with two pairs of shRNA specifically targeting HIF-1α (shR-α14 and shR-α16) and a negative control (NC) shRNA. shR-α14 and shR-α16, but not the negative control, significantly eliminated ATRA-stabilized HIF-1a protein but failed to affect HIF-1β expression (Figure 2A). Under the interference of these specific shRNA, the differentiation of U937 induced by 10^{-6}M of ATRA was partially inhibited (Figure 2B and C). As previously reported,[25,26] ATRA treatment up-regulated M-CSFR and G-CSFR, but not GM-CSFR,

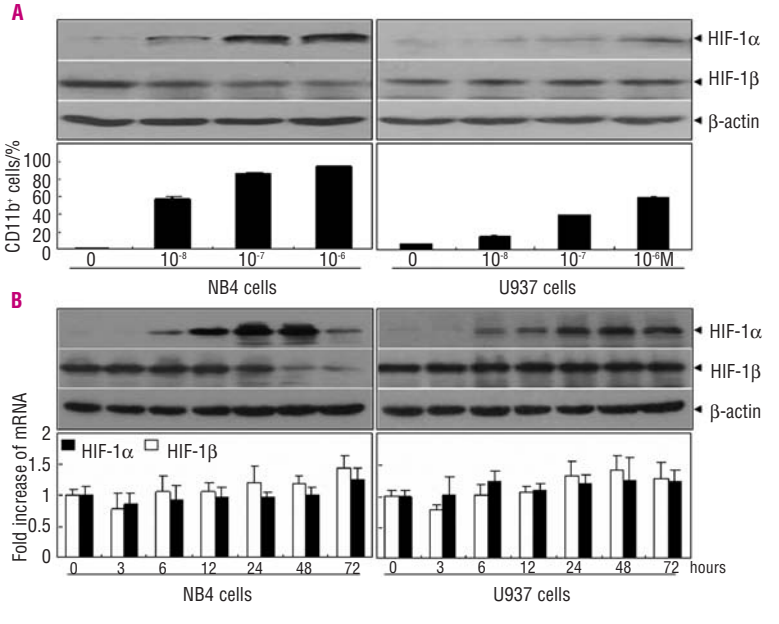

Figure 1. ATRA increases HIF-1α protein in leukemic cells. (A) NB4 and U937 cells were treated with the indicated concentrations of ATRA for 24 hours to test HIF-1α and HIF-1β proteins with β-actin as a loading control, or for 3 days to measure CD11b$^+$ cells % by flow cytometry. (B) NB4 and U937 cells were incubated with 10^{-6}M of ATRA for the number of hours indicated, and HIF-1α/HIF-1β protein and mRNA were evaluated by western blots and real-time quantitative RT-PCR, respectively. All experiments were repeated five times with similar results.

expression. More interestingly, the suppression of HIF-1α expression by its specific shRNA greatly inhibited the M-CSFR expression induced by ATRA (Figure 2D).

ATRA enhances inducible HIF-1α protein-triggered differentiation of leukemic cells

As reported previously,[19] conditional HIF-1α induction triggers differentiation of transformed U937T myeloid leukemic cells, including U937T^{clone} and U937T^{pool}, in which HIF-1α is induced by the withdrawal of tetracycline from the cell cultures. A low concentration (10^{-8}M) of ATRA also significantly increased the accumulation of HIF-1α protein induced by tetracycline withdrawal in both U937T^{clone} and U937T^{pool} (Figure 3A). Of note, the levels of HIF-1β protein remained stable during these treatments. As expected, 10^{-8}M of ATRA also significantly enhanced conditional HIF-1α induction-triggered differentiation, as assessed by morphological features (Figure 3B), and significantly increased CD11b^{+}% (Figure 3C) and M-CSFR mRNA expression (Figure 3D) in these two transformed cell lines.

ATRA and $CoCl_2$ cooperate to accumulate HIF-1α protein and induce differentiation in leukemic cells

We also investigated whether ATRA enhances the HIF-1α stabilization induced by hypoxia-mimetic agents. For this purpose, NB4 and U937 cells were treated with 10^{-8}M of ATRA together with 50 μM of $CoCl_2$. This low concentration of ATRA did increase the levels of $CoCl_2$-stabilized HIF-1α protein in NB4 and U937 cells (Figure 4A). It is worth noting that $CoCl_2$ could rescue the decreased expression of HIF-1β protein induced by ATRA in NB4 cells (Figure 4A). Furthermore, a single administration of 50 μM of $CoCl_2$ or 10^{-8}M of ATRA induced these two cell lines to undergo differentiation to a degree consistent with previous reports.[27,28] Combined treatment with these two agents obviously enhanced differentiation of NB4 and U937 cells (Figure 4B). The combined treatment also cooperatively induced expression of M-CSFR but not G-CSFR or GM-CSFR mRNA (Figure 4C). Although $CoCl_2$ did not increase the NBT response, it significantly enhanced the percentage of ATRA-induced NBT-positive NB4 cells (Figure 4D).

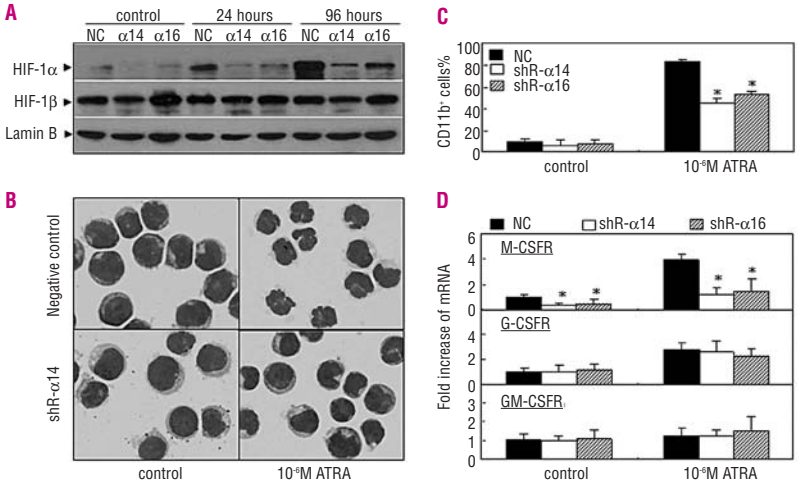

Figure 2. Silencing of HIF-1α expression by shRNA partially suppresses ATRA-induced differentiation of leukemic U937 cells. The U937 cell line, stably transfected by shR-α14, shR-α16 and a negative control (NC), were treated or not with 10^{-6}M of ATRA for 24 hours (A) or 96 hours (A-D). For the morphological characterization (B), all cells were stained with Wright's stain and observed under a light microscope (100×/1.30 Oil objective lens). For mRNA levels, the fold increases against NC-transfected control cells were calculated. The symbol * indicates a *p* value of less than 0.001 compared with the NC-transfected cells. All values represent means, with standard deviations shown by bars, of three independent experiments, each on triplicate samples.

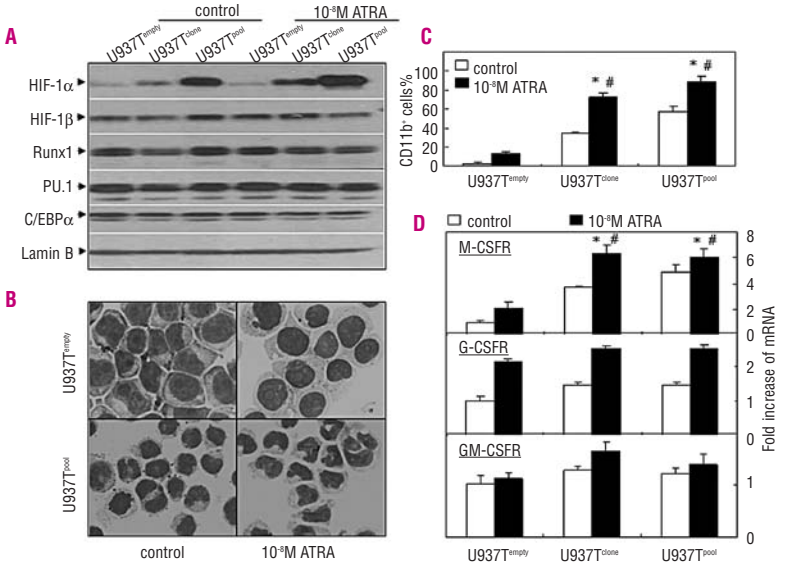

Figure 3. A low concentration of ATRA enhances the differentiation induced by conditioned HIF-1α. U937T^{clone}, U937T^{pool} and U937T^{empty} cells were grown in tetracycline-free medium with or without 10^{-8}M of ATRA for 24 hours (A) or 3 days (B-D). Then, the indicated proteins (A), cell morphology (B), CD11b^{+} cells % (C), and mRNA levels of M-CSFR, G-CSFR and GM-CSFR (D) were measured. For mRNA levels, the fold increases relative to untreated U937T^{empty} cells were calculated. The symbol * indicates a *p* value of less than 0.001 against the U937T^{empty} cell line. All values represent means, with standard deviations shown by bars, of three independent experiments, each with triplicate samples.

Specific interference of C/EBPα, Runx1 and PU.1 significantly reduced HIF-1α-triggered differentiation and its cooperation with ATRA

As mentioned above, C/EBP, Runx1 and PU.1 play important roles in hematopoietic cell differentiation and ATRA-induced differentiation of AML cells.[13,14] Furthermore, we previously showed that HIF-1α protein can physically interact with and enhance the transcriptional activity of C/EBPα[17,21] and Runx1.[20] Therefore, although conditional HIF-1α expression and $CoCl_2$ treatment failed to change the levels of these proteins regardless of the presence of 10^{-8}M ATRA (Figure 3A and 4A), we still attempted to understand the potential role of these transcriptional factors in the cooperative differentiation-inducing effect of HIF-1α with ATRA. To this purpose, shRNA specifically targeting PU.1, Runx1 and C/EBPα (named shR-P3, shR-A2 and shR-C2, respectively) were transfected into leukemic U937T[clone] cells. An empty vector was transfected as a negative control. These shRNA effectively knocked down expression of their cognate targeted genes (Figure 5A). The cells were then incubated with or without 10^{-8}M of ATRA for 3 days in the presence or absence of tetracycline. As depicted in Figure 5B, the suppression of expression of all three transcriptional factors (PU.1, Runx1 and C/EBPα) also partially blocked inducible HIF-1α expression-triggered myeloid cell differentiation. More intriguingly, the differentiation induced by the cooperation of ATRA and HIF-1α was also significantly inhibited by the suppression of expression of these hematopoietic transcriptional factors.

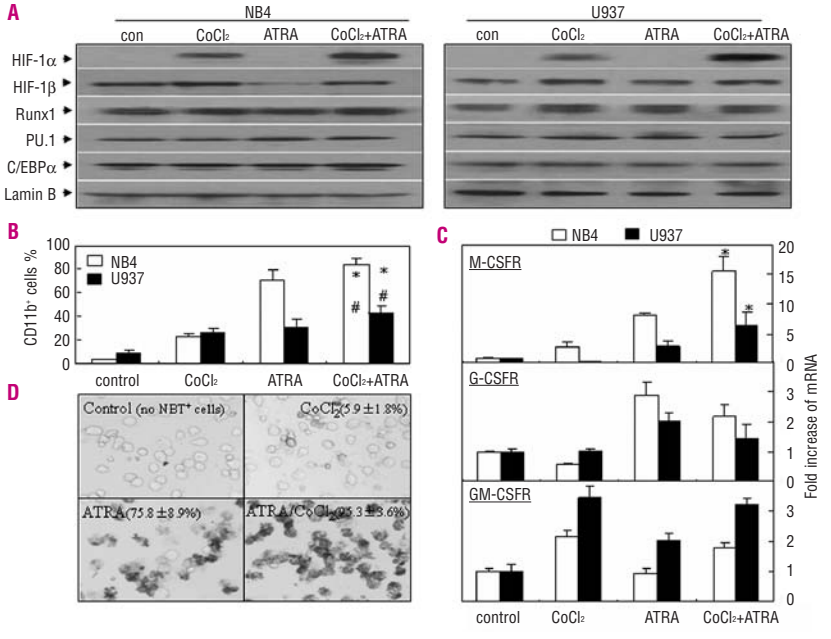

Figure 4. ATRA and $CoCl_2$ cooperate to accumulate HIF-1α protein and to induce differentiation of AML cell lines. NB4 cells and U937 cells were cultured with 50 μM $CoCl_2$ and/or 10^{-8}M ATRA for 24 hours before the measurement of the indicated proteins (A), or for 4 days to evaluate $CD11b^+$ cells % (B), mRNA levels of M-CSFR, G-CSFR and GM-CSFR (C) and NBT reduction (D, for NB4 cells). For mRNA levels, fold increases relative to untreated cells were calculated. The symbols * and # represent $p<0.01$ compared with $CoCl_2$ and ATRA alone, respectively. All values represent means, with standard deviations shown by bars, of four independent experiments, each with triplicate samples.

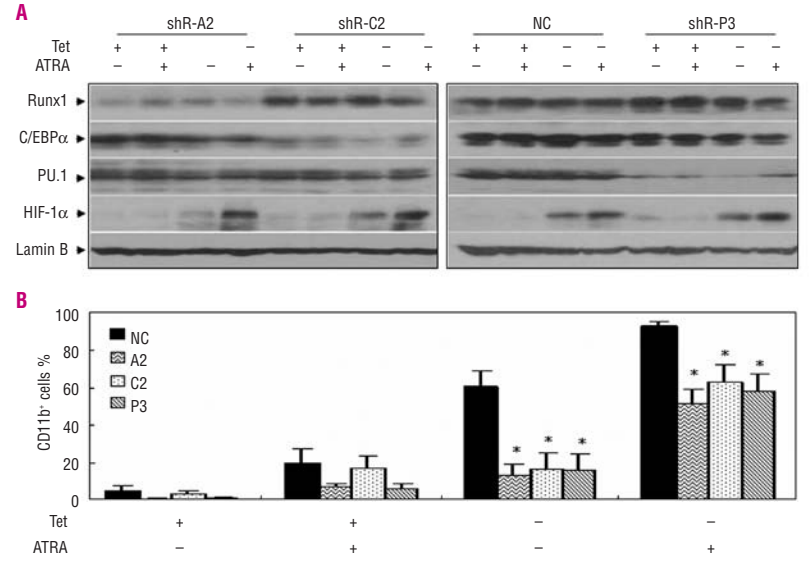

Figure 5. Specific suppression of Runx1, C/EBPα and PU.1 significantly prevents HIF-1α-triggered differentiation as well as its cooperation with ATRA. U937T[clone] cells were stably transfected with a negative control (NC) or shRNA against Runx1 (shR-A2), C/EBPα (shR-C2), or PU.1 (shR-P3). The transfected cells were then incubated in the presence or absence of tetracycline and/or 10^{-8}M of ATRA for 24 hours before blotting proteins as indicated (A) and for 3 days to detect $CD11b^+$% cells (B).

Discussion

As well documented,[15] the overall activity of HIF-1 is determined by the level of intracellular HIF-1α. In the past decade, the mechanisms of HIF-1α regulation have become a field of intense research and much information concerning it has been gained. In this study, we show that differentiation-inducing concentrations of ATRA rapidly increase HIF-1α protein but not its mRNA during the induced differentiation of both NB4 and U937 cells. ATRA also significantly increased the accumulation of HIF-1α protein induced by tetracycline withdrawal in engineered U937 cells, in which expression of HIF-1α mRNA was not under the control of the endogenous promoter of HIF-1α gene. Of note, NB4 and U937 cells presented different dynamic courses of changes in HIF-1α protein levels: the increase of HIF-1α protein was rapid but transitory in ATRA-treated NB4 cells, while ATRA induced a lasting increase of HIF-1α protein in U937 cells. All these observations indicate that ATRA accumulates HIF-1α protein via a post-transcriptional or translational mechanism. To date, several post-translational modifications of HIF-1α have been identified as regulating HIF-1 stability and activity, among which the most significant finding is the regulation by oxygen tension-dependent hydroxylation. Under normoxic conditions, proline residues 402 and 564 in the oxygen-dependent degradation domain of HIF-1α are hydroxylated by oxygen-activated HIF prolyl hydroxylases. This hydroxylation pushes HIF-1α toward ubiquitylation by E3 ubiquitin protein ligase von Hippel-Lindau tumor suppressor protein.[29] Ubiquitinylated HIF-1α is then rapidly degraded by proteasomes. Under hypoxic or hypoxia-mimetic conditions, HIF-1α protein accumulates thanks to the significantly reduced enzymatic activities of the prolyl hydroxylases. In addition, acetylation of a lysine residue (Lys532) by ARD1 acetyl transferase also enhances the binding of HIF-1α to von Hippel-Lindau protein and its subsequent degradation.[30] The S-nitrosylation of the HIF-1α protein at Cys533 in the oxygen-dependent degradation domain also prevents its destruction.[31] Moreover, sentrin/SUMO-specific protease1 (SENP1) plays a key role in the regulation of HIF1α stability.[32] Although the effects of ATRA on the production of nitric oxide, a donor for nitrosylation, are controversial,[33,34] we still investigated whether the S-nitrosylation or deSUMOylation of HIF-1α protein contributes to the ATRA-induced increase of HIF-1α protein. We found that L-NAME and 1400W, potent inhibitors of nitric oxide synthases,[31] failed to inhibit the ATRA-induced increase of HIF-1α protein in complete culture medium, while SENP1 expression was slightly upregulated during ATRA treatment (*data not shown*). It, therefore, remains to be determined how ATRA stabilizes HIF-1α protein through influencing these post-translational mechanisms.

In this study we focused on investigating whether increased HIF-1α levels contribute to ATRA-induced differentiation of AML cells. Although some reports have suggested that U937 cells are unable to exhibit granulocytic differentiation,[14,35] this cell line has been employed to investigate the mechanism of ATRA-induced differentiation in many studies.[36-41] The benefit of U937 cells is that they are easy to manipulate genetically, for example by RNA interference and overexpression of genes of interest. Based on these considerations, we chose U937 cells as a model to investigate the mechanism of ATRA-induced differentiation. Our results showed that suppression of HIF-1α expression by specific shRNA partially but significantly inhibited ATRA-induced differentiation of U937 cells. Reciprocally, the differentiation-inducing effect of a low dose of ATRA was significantly enhanced in the presence of conditional HIF-1α induction in transfected leukemic U937T cells, suggesting that ATRA and HIF-1α protein co-operate in terms of differentiation induction. This notion is supported by the facts that $CoCl_2$ potentiated ATRA-induced differentiation of leukemic NB4 and U937 cells, and that this was concomitant with the enhancement of $CoCl_2$-induced accumulation of HIF-1α protein by ATRA. All these results indicate that HIF-1α protein exerts a role in the ATRA-induced differentiation of AML cells. Together with previous findings,[17,19,22,27] these results also suggest that HIF-1α protein may play a common role in leukemic cell differentiation. In line with this, dysregulated expression or genetic alterations of HIF-1 have been found in some AML.[42,43] It should be pointed out that phorbol-12-myristate 13-acetate (PMA) was reported to induce increased HIF-1α protein synthesis, but was dispensable in PMA-induced macrophage differentiation of leukemic THP1 cells.[44,45] This discrepancy supported the previous conclusion that different transcriptional factors or signaling molecules are involved in myeloid differentiation.[46]

In a previous study we showed that the role of HIF-1α protein in myeloid cell differentiation is independent of its transcriptional activity, because the knockdown of expression of HIF-1β failed to affect HIF-1α-mediated differentiation;[19] HIF-1α protein interacts physically with and enhances the transcriptional activity of C/EBPα and Runx1 proteins and these interactions in turn inhibit the transcriptional activity of HIF-1.[20,21] The roles of C/EBPα, Runx1 and PU.1 in hematopoiesis have been widely investigated. For example, conditioned expression of PU.1 in human APL cells was sufficient to trigger neutrophil differentiation, whereas a reduction of PU.1 caused by shRNA blocked ATRA-induced neutrophil differentiation.[14] Here we showed that suppression of expression of C/EBPα, Runx1 and PU.1 greatly inhibited HIF-1α-induced differentiation and its cooperative effects with ATRA. Combined with our previous findings,[20,21] our results suggest that HIF-1α protein can act as an adaptor protein that recruits C/EBPα, Runx1 and/or PU.1 into promoters of differentiation-related target genes. Interestingly, HIF-1β and C/EBPα compete with each other for direct binding to HIF-1α protein.[21] More intriguingly, ATRA also decreased HIF-1β protein in dose- and time-dependent manners in NB4 cells, but not in U937 cells. This could partially explain why

NB4 cells are more sensitive than U937 cells to ATRA-induced differentiation.

Authorship and Disclosures

JZ: performed most of the experiments and drafted the article; L-PS and YH: contributed to shRNA-related work; QZ, K-WZ: contributed to analysis of the data; G-QC: conception and design of the study, critical revision of the paper for important intellectual content and final approval of the version to be published. The authors reported no potential conflicts of interest.

References

1. Hansen LA, Sigman CC, Andreola F, Ross SA, Kelloff GJ, De Luca LM. Retinoids in chemoprevention and differentiation therapy. Carcinogenesis 2000;21:1271-9.
2. Tallman MS, Nabhan C, Feusner JH, Rowe JM. Acute promyelocytic leukemia: evolving therapeutic strategies. Blood 2002;99:759-67.
3. Melnick A, Licht JD. Deconstructing a disease: RARa, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. Blood 1999;93: 3167-215.
4. Kambhampati S, Verma A, Li Y, Parmar S, Sassano A, Platanias LC. Signalling pathways activated by all-trans-retinoic acid in acute promyelocytic leukemia cells. Leuk Lymphoma 2004;45:2175-85.
5. Wolf G, Smas CM. Retinoic acid induces the degradation of the leukemogenic protein encoded by the promyelocytic leukemia gene fused to the retinoic acid receptor a gene. Nutr Rev 2000;58:211-4.
6. Grignani F, De Matteis S, Nervi C, Tomassoni L, Gelmetti V, Cioce M, et al. Fusion proteins of the retinoic acid receptor-α recruit histone deacetylase in promyelocytic leukaemia. Nature 1998;391:815-8.
7. Harris MN, Ozpolat B, Abdi F, Gu S, Legler A, Mawuenyega KG, et al. Comparative proteomic analysis of all-trans-retinoic acid treatment reveals systematic posttranscriptional control mechanisms in acute promyelocytic leukemia. Blood 2004;104:1314-23.
8. Pitha-Rowe I, Petty WJ, Kitareewan S, Dmitrovsky E. Retinoid target genes in acute promyelocytic leukemia. Leukemia 2003;17:1723-30.
9. Zhao Q, Tao J, Zhu Q, Jia PM, Dou AX, Li X, et al. Rapid induction of cAMP/PKA pathway during retinoic acid-induced acute promyelocytic leukemia cell differentiation. Leukemia 2004;18:285-92.
10. Glasow A, Prodromou N, Xu K, von Lindern M, Zelent A. Retinoids and myelomonocytic growth factors cooperatively activate RARA and induce human myeloid leukemia cell differentiation via MAP kinase pathways. Blood 2005; 105:341-9.
11. Tenen DG, Hromas R, Licht JD, Zhang DE. Transcription factors, normal myeloid development, and leukemia. Blood 1997;90:489-519.
12. Rosenbauer F, Tenen DG. Transcription factors in myeloid development: balancing differentiation with transformation. Nat Rev Immunol 2007;7:105-17.
13. Duprez E, Wagner K, Koch H, Tenen DG. C/EBPb: a major PML-RARA-responsive gene in retinoic acid-induced differentiation of APL cells. Embo J 2003;22:5806-16.
14. Mueller BU, Pabst T, Fos J, Petkovic V, Fey MF, Asou N, et al. ATRA resolves the differentiation block in t(15;17) acute myeloid leukemia by restoring PU.1 expression. Blood 2006;107:3330-8.
15. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci USA 1995;92:5510-4.
16. Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003;3:721-32.
17. Jiang Y, Xue ZH, Shen WZ, Du KM, Yan H, Yu Y, et al. Desferrioxamine induces leukemic cell differentiation potentially by hypoxia-inducible factor-1α that augments transcriptional activity of CCAAT/enhancer-binding protein-α. Leukemia 2005;19:1239-47.
18. Liu W, Guo M, Xu YB, Li D, Zhou ZN, Wu YL, et al. Induction of tumor arrest and differentiation with prolonged survival by intermittent hypoxia in a mouse model of acute myeloid leukemia. Blood 2006;107:698-707.
19. Song LP, Zhang J, Wu SF, Huang Y, Zhao Q, Cao JP, et al. Hypoxia-inducible factor-1α-induced differentiation of myeloid leukemic cells is its transcriptional activity independent. Oncogene 2008;27:519-27.
20. Peng ZG, Zhou MY, Huang Y, Qiu JH, Wang LS, Liao SH, et al. Physical and functional interaction of Runt-related protein 1 with hypoxia-inducible factor-1α. Oncogene 2008;27:839-47.
21. Yang L, Jiang Y, Wu SF, Zhou MY, Wu YL, Chen GQ. CCAAT/enhancer-binding protein alpha antagonizes transcriptional activity of hypoxia-inducible factor 1α with direct protein-protein interaction. Carcinogenesis 2008; 29:291-8.
22. Kim JS, Cho EW, Chung HW, Kim IG. Effects of Tiron, 4,5-dihydroxy-1,3-benzene disulfonic acid, on human promyelotic HL-60 leukemia cell differentiation and death. Toxicology 2006;223:36-45.
23. Zhu J, Shi XG, Chu HY, Tong JH, Wang ZY, Naoe T, et al. Effect of retinoic acid isomers on proliferation, differentiation and PML relocalization in the APL cell line NB4. Leukemia 1995;9:302-9.
24. Zhao KW, Li X, Zhao Q, Huang Y, Li D, Peng ZG, et al. Protein kinase Cd mediates retinoic acid and phorbol myristate acetate-induced phospholipid scramblase 1 gene expression: its role in leukemic cell differentiation. Blood 2004;104: 3731-8.
25. Hsu HC, Yang K, Kharbanda S, Clinton S, Datta R, Stone RM. All-trans retinoic acid induces monocyte growth factor receptor (c-fms) gene expression in HL-60 leukemia cells. Leukemia 1993;7:458-62.
26. Tkatch LS, Rubin KA, Ziegler SF, Tweardy DJ. Modulation of human G-CSF receptor mRNA and protein in normal and leukemic myeloid cells by G-CSF and retinoic acid. J Leukoc Biol 1995;57:964-71.
27. Huang Y, Du KM, Xue ZH, Yan H, Li D, Liu W, et al. Cobalt chloride and low oxygen tension trigger differentiation of acute myeloid leukemic cells: possible mediation of hypoxia-inducible factor-1α. Leukemia 2003;17:2065-73.
28. Lanotte M, Martin-Thouvenin V, Najman S, Balerini P, Valensi F, Berger R. NB4, a maturation inducible cell line with t(15;17) marker isolated from a human acute promyelocytic leukemia (M3). Blood 1991;77:1080-6.
29. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 1999;399:271-5.
30. Jeong JW, Bae MK, Ahn MY, Kim SH, Sohn TK, Bae MH, et al. Regulation and destabilization of HIF-1a by ARD1-mediated acetylation. Cell 2002;111:709-20.
31. Li F, Sonveaux P, Rabbani ZN, Liu S, Yan B, Huang Q, et al. Regulation of HIF-1α stability through S-nitrosylation. Mol Cell 2007;26:63-74.
32. Cheng J, Kang X, Zhang S, Yeh ET. SUMO-specific protease 1 is essential for stabilization of HIF1a during hypoxia. Cell 2007;131:584-95.
33. Devaux Y, Grosjean S, Seguin C, David C, Dousset B, Zannad F, et al. Retinoic acid and host-pathogen interactions: effects on inducible nitric oxide synthase in vivo. Am J Physiol Endocrinol Metab 2000; 279:E1045-53.

34. Dugas N, Mossalayi MD, Calenda A, Leotard A, Becherel P, Mentz F, et al. Role of nitric oxide in the antitumoral effect of retinoic acid and 1,25-dihydroxyvitamin D3 on human promonocytic leukemic cells. Blood 1996;88:3528-34.
35. Grignani F, Ferrucci PF, Testa U, Talamo G, Fagioli M, Alcalay M, et al. The acute promyelocytic leukemia-specific PML-RARα fusion protein inhibits differentiation and promotes survival of myeloid precursor cells. Cell 1993; 74:423-31.
36. Svedberg H, Chylicki K, Baldetorp B, Rauscher FJ 3rd, Gullberg U. Constitutive expression of the Wilms' tumor gene (WT1) in the leukemic cell line U937 blocks parts of the differentiation program. Oncogene 1998;16:925-32.
37. Matikainen S, Ronni T, Hurme M, Pine R, Julkunen I. Retinoic acid activates interferon regulatory factor-1 gene expression in myeloid cells. Blood 1996;88:114-23.
38. Liu M, Iavarone A, Freedman LP. Transcriptional activation of the human p21(WAF1/CIP1) gene by retinoic acid receptor. Correlation with retinoid induction of U937 cell differentiation. J Biol Chem 1996; 271:31723-8.
39. el Yaagoubi M, Hachad H, Leh H, Siest G, Wellman M. Gamma-glutamyltransferase expression during all-trans retinoic acid-induced differentiation of hematopoietic cell lines. FEBS Lett 1995;369:183-6.
40. El Marjou M, Montalescot V, Buzyn A, Geny B. Modifications in phospholipase D activity and isoform expression occur upon maturation and differentiation in vivo and in vitro in human myeloid cells. Leukemia 2000;14:2118-27.
41. Dimberg A, Nilsson K, Oberg F. Phosphorylation-deficient Stat1 inhibits retinoic acid-induced differentiation and cell cycle arrest in U-937 monoblasts. Blood 2000;96: 2870-8.
42. Yuan W, Payton JE, Holt MS, Link DC, Watson MA, DiPersio JF, et al. Commonly dysregulated genes in murine APL cells. Blood 2007;109: 961-70.
43. Nguyen-Khac F, Della Valle V, Lopez RG, Ravet E, Mauchauffe M, Friedman AD, et al. Functional analyses of the TEL-ARNT fusion protein underscores a role for oxygen tension in hematopoietic cellular differentiation. Oncogene 2006; 25:4840-7.
44. Knowles HJ, Mole DR, Ratcliffe PJ, Harris AL. Normoxic stabilization of hypoxia-inducible factor-1α by modulation of the labile iron pool in differentiating U937 macrophages: effect of natural resistance-associated macrophage protein 1. Cancer Res 2006;66:2600-7.
45. Oda T, Hirota K, Nishi K, Takabuchi S, Oda S, Yamada H, et al. Activation of hypoxia-inducible factor 1 during macrophage differentiation. Am J Physiol Cell Physiol 2006;291:C104-13.
46. Lenny N, Westendorf JJ, Hiebert SW. Transcriptional regulation during myelopoiesis. Mol Biol Rep 1997;24:157-68.